Nanosphere

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Summary of 510(k) Safety and Effectiveness

This summary of the 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1900 and CFR 807.92.

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Proprietary names:
For instrument:
Verigene® System

For the assay:
Verigene® Respiratory Virus Nucleic Acid Test

Common names:
For the instrument:
Bench-top molecular diagnostics workstation

For the assays:
Respiratory panel
Respiratory virus panel
Respiratory viruses
Influenza A assay
Influenza B assay
RSV assay
Influenza A/B and RSV assay

Device descriptions:
The Verigene Respiratory Virus Nucleic Acid Test (VRNAT) is based on uniquely identifying virus-specific nucleic acids for Influenza A virus, Influenza B virus, and Respiratory Syncytial Virus (RSV). The VRNAT involves the following steps: (i) Sample Preparation: Isolation of the viral RNA from nasopharyngeal swab specimens obtained from symptomatic patients. Sample preparation is conducted on an automated sample isolation system (NucliSens® easyMAG™ System, bioMérieux); (ii) Multiplex RT-PCR procedure for the generation of virus-specific amplicons: A specified volume of the eluted genomic RNA from the sample preparation step is subjected to an RT-PCR target

amplification step; (iii) Verigene Test: Conducted on the Verigene® System for detection and identification of virus-specific amplicons.

The Verigene System consists of two instruments, the *Verigene Processor* and the *Verigene Reader*, and utilizes single-use, disposable *Test Cartridges*.

The *Verigene Reader* is a bench-top, free-standing instrument with a touch screen control panel and a wand-based barcode scanner. It utilizes a graphical user interface to guide the user through the process of ordering tests and reporting results. There are no serviceable parts and no user calibration is required. Interaction with the touch screen is minimized through barcode use. This instrument also serves as the reader of the hybridization substrate using optical detection.

The key functions of the Verigene Reader include:

- Entry and tracking of specimen identification numbers via manual keyboard input or via barcode-reader wand.
- Test selection for each specimen.
- Automated transfer of specimen processing instructions on Test Cartridge-specific basis to linked Processor unit(s).
- Automated imaging and analysis of Test Cartridges.
- Results display.
- Results report generation.

The *Verigene Processor* performs the Verigene Test under the direction of the Verigene Reader. It has been designed to be simple and easy to use with minimal user interaction. It contains no fluids and has no user calibration requirements. There are four hybridization modules in each Verigene Processor and up to eight Verigene Processors may be connected to a single Verigene Reader. The modules within a Verigene Processor can simultaneously run different tests. When a *Test Cartridge* containing the sample mix is inserted, a barcode reader internal to the Verigene Processor modules reads the cartridge ID and sends it to the Verigene Reader. From this information the Verigene Reader establishes the hybridization parameters and automatically starts the Verigene Test.

The *Test Cartridge* consists of two parts: a Reagent Pack with reservoirs preloaded with test-specific reagents, and a Substrate Holder. The reagent pack creates an air-tight hybridization chamber surrounding the region of the substrate-containing target-specific capture array. As each step in the Verigene Test is completed, old reagents are moved out of the hybridization chamber and new reagents are added from the reagent pack via microfluidic channels and pumps.

To run the Verigene Test, the user mixes the sample with a test-specific Sample Buffer and loads this sample mix into the Test Cartridge. The Test Cartridge is subsequently inserted into the Processor. Both the identity of the test contained in the Test Cartridge and the associated patient specimen are linked with a common barcode that is unique to each Test Cartridge. Test information entered at the beginning of each test session is used to direct the associated module of the Processor unit on what steps to execute to process the Test Cartridge, and the patient specimen information is used for tracking and results reporting. Once the test is complete, the Test Cartridge is removed from the Processor unit and the reagent pack is snapped off and discarded. The remaining Test Substrate is now ready for imaging and analysis in the Reader unit.

The Test Substrate is inserted into the Reader wherein it is illuminated along its side. The gold-silver aggregates at the test sites scatter the light, which is in turn captured by a photosensor. The relative intensity arising from each arrayed test site is tabulated. Net signals, defined as the absolute signal intensities with background signals subtracted, are compared with thresholds determined by negative and positive controls within the slide in order to arrive at a decision regarding the presence or absence of target. These results are linked to the test and patient information entered at the beginning of each test session to provide a comprehensive results file.

Intended uses:

The Verigene® Respiratory Virus Nucleic Acid Test is a qualitative multiplex *in vitro* diagnostic test for the detection and identification of Influenza A Virus, Influenza B Virus, and Respiratory Syncytial Virus (RSV) nucleic acids purified from nasopharyngeal swab

specimens obtained from patients symptomatic for viral upper respiratory infection. The test is intended to be used on the Verigene® System as an aid in the differential diagnosis of Influenza A, Influenza B, and RSV infections. The test is not intended to detect Influenza C virus.

Negative results do not preclude influenza virus or RSV infection and should not be used as the sole basis for treatment or other management decisions. It is recommended that negative test results be confirmed by culture.

Performance characteristics for Influenza A Virus were established when Influenza A/H3 and A/H1 were the predominant Influenza A viruses in circulation. When other Influenza A viruses are emerging, performance characteristics may vary.

If infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent Influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

Predicate device:

The Prodesse ProFlu+™ Assay [K073029 and K081030] is claimed as the predicate device for the Verigene® Respiratory Virus Nucleic Acid Test.

The ProFlu+ Assay is a multiplex Real Time RT-PCR *in vitro* diagnostic test for the rapid and qualitative detection and discrimination of Influenza A Virus, Influenza B Virus, and Respiratory Syncytial Virus (RSV) nucleic acids isolated and purified from nasopharyngeal (NP) swab specimens obtained from symptomatic patients. This test is intended for use to aid in the differential diagnosis of Influenza A, Influenza B, and RSV viral infections in humans and is not intended to detect Influenza C virus.

Negative results do not preclude influenza virus or RSV infection and should not be used as the sole basis for treatment or other management decisions. It is recommended that negative RSV results be confirmed by culture.

The tables below compare the Verigene Respiratory Virus Nucleic Acid Test with the predicate device:

Similarities to predicate device

Feature	Verigene Respiratory Virus Nucleic Acid Test	Prodesse ProFlu+ Assay
Intended use	The Verigene Respiratory Virus Nucleic Acid Test is a qualitative *in vitro* diagnostic for the identification of Influenza A virus, Influenza B virus, and Respiratory Syncytial Virus (RSV) in nasopharyngeal swab specimens as an aid in the differential diagnosis of patients symptomatic for viral upper respiratory infection. The test is not intended to detect Influenza C virus. The test is intended to be used on the Verigene System. Negative results do not preclude influenza virus or RSV infection and should not be used as the sole basis for treatment or other management	The ProFlu+ Assay is a multiplex Real Time RT-PCR *in vitro* diagnostic test for the rapid and qualitative detection and discrimination of Influenza A Virus, Influenza B Virus, and Respiratory Syncytial Virus (RSV) nucleic acids isolated and purified from nasopharyngeal (NP) swab specimens obtained from symptomatic patients. This test is intended for use to aid in the differential diagnosis of Influenza A, Influenza B and RSV viral infections in humans and is not intended to detect Influenza C. Negative results do not preclude

	decisions. It is recommended that negative RSV results be confirmed by culture.	influenza or RSV virus infection and should not be used as the sole basis for treatment or other management decisions. It is recommended that negative RSV results be confirmed by culture.
Targets	Influenza A Influenza B RSV	Influenza A Influenza B RSV
Specimen	Nasopharyngeal swabs in sample matrix	Nasopharyngeal swabs in sample matrix
Sample preparation	Automated extraction of nucleic acids	Automated extraction of nucleic acids
Sample size	200 µL	200 µL
Quality control	Internal procedural quality controls including a process control and an inhibition control and external quality control solutions	Internal procedural quality control, external quality control solutions
Amplification method	Multiplex RT-PCR	Multiplex RT-PCR
	M-MLV Reverse Transcriptase	M-MLV Reverse Transcriptase
Results	Positive or negative qualitative results	Positive or negative qualitative results

Differences from predicate device

Feature	Verigene Respiratory Virus Nucleic Acid Test	Prodesse ProFlu Assay
Amplification method	Tfi polymerase	Taq polymerase
Contamination control	Thermolabile Uracil DNA Glycosylase (UDG) to limit amplicon contamination	None
Test principle	Gold nanoparticle probe-based chemistry	Taqman chemistry
Detection method	End-point detection of the amplified material on the Verigene System	Real-time RT-PCR
	Single-image sensor where nanoparticles are illuminated using a fixed-wavelength light source	Fluorescence-based detection
	Microarray format: vastly greater multiplex capability. Allows for greater coverage of strain variants within the viruses.	Real-time detection format: limited multiplex capability.
Reagent storage	Test cartridges and Sample Buffer stored at 2 – 8 °C. Amplification kit stored at -20 °C. Controls stored at -20 °C.	All reagents (opened and unopened) stored at -70 °C.
Quality control	Two internal procedural controls: (i) Process Control – controls for sample isolation and the RT-PCR steps. (ii) Inhibition Control – controls for the amplification step.	Single internal procedural control.

Performance Characteristics

A. Clinical Performance

A total of 720 nasopharyngeal swab specimens were prospectively collected during the 2007-2008 respiratory season for routine influenza or RSV testing by DFA/culture methods. The residual specimens were frozen and later tested at three clinical sites, 240 at each site, using the Verigene® Respiratory Virus Nucleic Acid Test. The patient population was distributed by age as presented in the table below:

Patient Population by Age	Number of Subjects
0 – 1	120
1 – 5	229
5 – 20	129
20 – 65	204
>65	38
Total	720

Samples with an initial 'No Call' result were re-tested successfully by following the recommendations in the 'Results Interpretation' section (see above). A total of 21 samples (2.9%) generated a "No Call" result; ten (10) of the samples (1.4%) gave a 'No Call – Indeterminate Zone' result indicating an equivocal call. Repeat testing resolved each of these samples. Another eleven (11) samples (1.5%) gave a 'No Call – Pos Ctrl' call indicating an inability to detect an internal control. All the samples were resolved upon retest.

The VRNAT performance was compared to a culture-based reference method followed by direct fluorescent antibody (DFA) identification of all culture positive samples. The results for each target appear in the tables below. Discordant results between the VRNAT and the reference method were followed up by using bi-directional sequencing at an independent reference laboratory and described in footnotes.

Influenza A Results

All Sites Influenza A		DFA/Viral Culture			
		Positive	Negative	Total	
Verigene System	Positive	122	59 [a]	181	Sensitivity 99.2% (95%CI=95.5% - 99.9%)
	Negative	1 [b]	538	539	Specificity 90.1% (95%CI=87.5% - 92.3%)
	Total	123	597	720	

a. 58 samples were positive by sequencing. 1 sample was negative by sequencing.
b. 1 sample was negative by sequencing

Influenza B Results

All Sites Influenza B		DFA/Viral Culture			
		Positive	Negative	Total	
Verigene System	Positive	30	10 [a]	40	Sensitivity 96.8% (95%CI=83.5% - 99.4%)
	Negative	1 [b]	679	680	Specificity 98.5% (95%CI=97.3% - 99.2%)
	Total	31	689	720	

a. 4 samples were positive by sequencing. 6 samples were negative by sequencing
b. 1 sample was negative by sequencing

RSV Results

All Sites RSV		DFA/Viral Culture			
		Positive	Negative	Total	
Verigene System	Positive	44 [a]	57 [b]	101	Sensitivity 89.8% (95%CI=78.2% - 95.6%)
	Negative	5 [c]	614	619	Specificity 91.5% (95%CI=89.2% - 93.4%)
	Total	49	671	720	

a. All 44 samples were positive by sequencing.
b. 46 samples were positive by sequencing. 11 samples were negative by sequencing.
c. 4 samples were positive by sequencing. 1 sample was negative by sequencing.

Dual Infections

Of the 720 samples included in the clinical study, 12 samples were positive for 2 infections by the VRNAT, translating to 1.7% of the samples tested. Comparable RT-PCR—based real-time detection methods have reported a significantly lower percentage for dual infections (~0.1%). Two inter-related factors may contribute to the higher percentage of the dual infections observed in the VRNAT. Competitive inhibition is often observed in dual infections, especially when one infective agent is at a much lower initial concentration resulting thereby in amplicons below the detection limits of fluorescence-based methods.[i,ii,iii,iv] The Verigene System uses gold nanoparticle probe-based technology that is at least 2-3 log orders more sensitive than fluorescence-based methods[v].

B. Reproducibility & Precision

The reproducibility study was performed at each of three sites. At Site 1, the reproducibility study was part of a larger precision study (see below). Eight unique samples were created by diluting known concentrations of viral particles with Viral Transport Medium. The following strains representing Influenza A, Influenza B, RSV A, and RSV B were used to prepare the samples: Influenza A/Wisconsin/67/2005; Influenza B/ Florida/04/2006; RSV A Strain Long; RSV B Strain B-1 Wild Type (B WV/14617/85). Each strain was represented at 3 distinct concentrations: high negative, low positive, and moderate positive.

Unique Samples	Viral Strains and Levels
1	Influenza A - High Negative; Influenza B - High Negative
2	RSV A - High Negative; RSV B - High Negative
3	Influenza A - Low Positive
4	Influenza B - Low Positive
5	RSV A - Low Positive
6	RSV B - Low Positive
7	Influenza A - Moderate Positive; RSV A - Moderate Positive
8	Influenza B - Moderate Positive; RSV B - Moderate Positive

The precision study (Site 1) used the same set of 8 samples and was performed over 12 non-consecutive days. On each test day, two operators performed the VRNAT, involving sample isolation, target amplification, and the Verigene Test, on set of 8 samples in duplicate (i.e., 4 sample sets per day total). In the reproducibility study performed by sites 2 and 3, the samples were analyzed on the Verigene System in triplicate daily by 2 operators on each of five non-consecutive days after undergoing sample isolation and target amplification.

In the 'Reproducibility and Precision' studies, the moderate positive samples served as positive controls since they were expected to provide positive decisions 100% of the time. The same samples also served as negative controls; 'Not Detected' decisions were expected at Influenza B and RSV B in the moderate positive samples containing Influenza A and RSV A and conversely 'Not Detected' decisions were expected at Influenza A and

RSV A in the moderate positive samples containing Influenza B and RSV B. The positive and negative controls provided expected decisions during the studies. Calls made by the Verigene System were compared to the expected outcome as the measure of performance. The table below shows the summary results of the reproducibility study.

	Characteristic	Verigene Respiratory Virus Nucleic Acid Test			
		Influenza A	*Influenza B*	*RSV A*	*RSV B*
Total Reproducibility	High Negative	75/78 (96.2%)	78/78 (100%)	76/78 (97.4%)	74/78 (94.9%)
	95% CI	89.3%-98.7%	95.3%-100%	91.1%-99.3%	87.5%-98.0%
	Low Positive	77/78 (98.7%)	77/78 (98.7%)	77/78 (98.7%)	78/78 (100%)
	95% CI	93.1%- 99.8%	93.1%-99.8%	93.1%-99.8%	95.3%-100%
	Moderate Positive	78/78 (100%)	78/78 (100%)	78/78 (100%)	78/78 (100%)
	95% CI	95.3%-100%	95.3%-100%	95.3%-100%	95.3%-100%

C. Analytical Sensitivity

The analytical sensitivity was demonstrated by determining the limit of detection or LoD of the VRNAT by using strains with established titers for the following viruses: Influenza A, Influenza B, RSV A, and RSV B. Each virus stock was serially diluted into a sample matrix (Universal Transport Media, Copan). The dilution series was taken through the VRNAT process and tested in triplicate at each dilution for each virus. The limit of detection defined as the lowest concentration at which ≥95% of the replicates tested positive, was assessed based on the performance of the samples within each dilution series as judged by the Verigene calls. This LoD was confirmed by performing an additional 20 replicates for each strain in order to demonstrate that the virus was detected ≥95% of the time.

Limits of Detection	Concentration
Influenza A strains	
Influenza A/Wisconsin/67/05 (H3N2)	2 TCID50/mL
Influenza A/New Caledonia/20/99 (H1N1)	50 TCID50/mL
Influenza A/Port Chalmers/1/73 (H3N2)	50 TCID50/mL
Influenza B strains	
Influenza B/Florida/04/2006	60 TCID50/mL
Influenza B/Lee/40	0.01 EID50/mL
Influenza B/Hong Kong/5/72	0.05 EID50/mL
RSV A strains	
RSV A Strain A2	10 TCID50/mL
RSV A Strain Long	10 TCID50/mL
RSV B strains	
RSV B Strain B-1 Wild Type (B WV/14617/85)	2 TCID50/mL
RSV B Strain Wash/18537/62	0.5 TCID50/mL
RSV B Strain 9320	0.05 TCID50/mL

D. Determination of Clinical Cut-off and VRNAT Call Algorithm

The Verigene Respiratory Virus Nucleic Acid Test (VRNAT) uses a microarray-based platform in which the viruses and the inhibition control (PC1) and process control (PC2) are represented by recognition elements on the Test Substrate. The recognition elements or spots are virus-specific oligonucleotide sequences that bind to the amplified viral targets, which in turn bind to gold nanoparticle probes via additional recognition elements. A gold nanoparticle probe-specific signal enhancement reaction deposits silver at the virus-specific spots. The scatter from the spots is detected by the Verigene Reader and registered as signal intensity. In addition to the above recognition elements, the Test Substrate has spots specific to positive control (PC) and negative control (NC). Three conditions were identified that together served as a single set of clinical cutoff criteria.

Condition 1: Noise Threshold

Condition 2: Normalized 'Ratio to Negative Control' (Ratio-to-NC) – intensity at the virus-specific recognition element normalized against the intensity values at the negative control elements.

Condition 3: Normalized 'Ratio to Positive Control' (Ratio-to-PC) – intensity at the virus-specific recognition element normalized against the intensity values at the positive control elements.

The Noise Threshold was determined empirically to be equal to Negative Control + 2 SD. The Noise Threshold was determined empirically to be equal to Negative Control + 2 SD. The cut-offs for the normalized ratios, 'Ratio-to-NC' and the 'Ratio-to-PC', were determined by using ROC curves. For a positive 'Detected' decision the following criteria apply:

Condition 1: Signal intensity is above the noise threshold

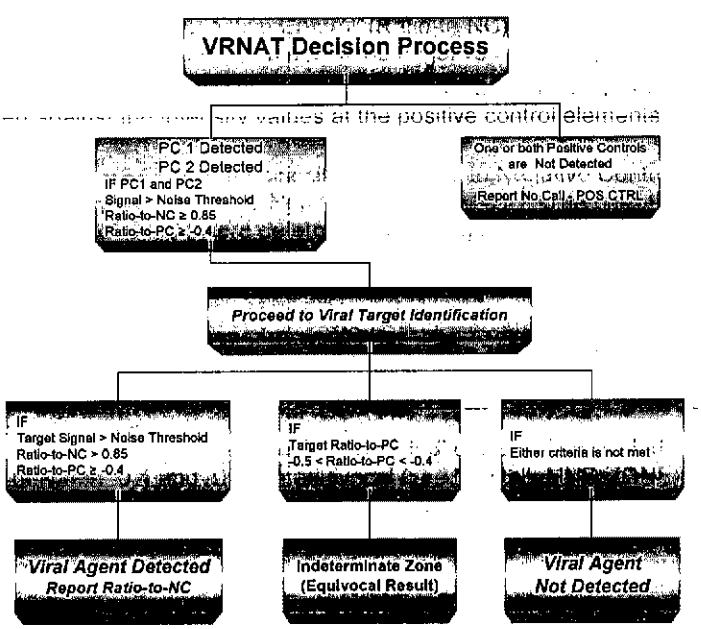
Condition 2: Ratio-to-NC is above 0.85

Condition 3: Ratio-to-PC is above -0.4

If any one of these criteria is not met, a negative 'Not Detected' decision is provided. If the Ratio-to-PC for any one of the three viruses falls between -0.4 and -0.5, an equivocal (i.e., No Call – IND ZONE) decision is provided. Criteria set for each of the three conditions are required to be met for a 'Detected' call.

VRNAT Decision Process

For a result, the decision tree examines the presence of both PC1 and PC2 initially (see Schematic). Both PC1 and PC2 signal intensities have to meet the detection criteria before the analysis is allowed to proceed toward establishing the presence or absence (i.e., "Detected" or "Not Detected") of the individual viruses.



E. Analytical Reactivity

The analytical reactivity (inclusivity) of the Verigene® Respiratory Virus Nucleic Acid Test was evaluated against strains of Influenza A, Influenza B, RSV A, and RSV B that are representative of temporal and geographical diversity. Known concentrations of the different viral strains grown in culture were diluted in sample matrix (Universal Transport Media, Copan) to achieve the titers listed (see Table below). A total of 14 strains for Influenza A were tested at 100 TCID50/mL (or EID50/mL). For Influenza B a total of 10 strains were tested at 100 TCID/mL. A total of 5 RSV strains were tested at 100 TCID50/mL for RSV A (2 strains) and RSV B (3 strains). Each strain was tested in triplicate at the said concentration by the VRNAT. All viral cultures were detected; the VRNAT analytical reactivity demonstrated 100% concordance with expected results.

Analytical Reactivity	Conc.	Influenza A	Influenza B	RSV
Influenza A strains				
Influenza A/Canada/578/04 (H3N2)	100 TCID50/mL	+	-	-
Influenza A/New Caledonia/20/99 (H1N1)	100 TCID50/mL	+	-	-
Influenza A/Hong Kong/29/2006 (H1N1)	100 TCID50/mL	+	-	-
Influenza A/Wisconsin/67/05 (H3N2)	100 TCID50/mL	+	-	-
Influenza A/PR/8/34 (H1N1)	100 EID50/mL	+	-	-
Influenza A/Aichi/68 (H3N2)	100 EID50/mL	+	-	-
Influenza A/WS/33 (H1N1)	100 EID50/mL	+	-	-
Influenza A/Port Chalmers/1/73 (H3N2)	100 EID50/mL	+	-	-
Influenza A/ Brisbane/59/ 2007 (H1N1)	100 EID50/mL	+	-	-
Influenza A/Brisbane/10/2007 (H3N2)	100 EID50/mL	+	-	-
Influenza A1/Denver/1/57 (H1N1)	100 EID50/mL	+	-	-
Influenza A1/FM/1/47 (H1N1)	100 EID50/mL	+	-	-
Influenza A/New Jersey/8/76 (H1N1)	100 EID50/mL	+	-	-
Influenza A/NWS/33 (H1N1)	100 EID50/mL	+	-	-
Influenza B strains				
Influenza B/Yamanashi/166/98	100 TCID50/mL	-	+	-
Influenza B/Panama/45/90	100 TCID50/mL	-	+	-
Influenza B/Florida/02/2006	100 TCID50/mL	-	+	-
Influenza B/Florida/04/2006	100 EID50/mL	-	+	-
Influenza B/ Lee/40	100 EID50/mL	-	+	-
Influenza B/Egypt/2040/04	100 TCID50/mL	-	+	-
Influenza B/GL/1739/54	100 EID50/mL	-	+	-
Influenza B/Hong Kong/5/72	100 EID50/mL	-	+	-
Influenza B/Maryland/1/59	100 EID50/mL	-	+	-
Influenza B/Taiwan/2/62	100 EID50/mL	-	+	-
RSV A strains				
RSV A Strain A2	100 TCID50/mL	-	-	+
RSV A Strain Long	100 TCID50/mL	-	-	+
RSV B strains				
RSV B Strain B-1 Wild Type (B WV/14617/85)	100 TCID50/mL	-	-	+
RSV B Strain Wash/18537/62	100 TCID50/mL	-	-	+
RSV B Strain 9320	100 TCID50/mL	-	-	+

F. Analytical Specificity/Cross-Reactivity

			Influenza A	Influenza B	RSV
Viruses	**Strain**	**pfu/mL**	-	-	-
Human Adenovirus Type 1	Adenoid 71	$3.1x10^5$	-	-	-
Human Adenovirus Type 7	Gomen	$3.1x10^5$	-	-	-
Human coronavirus (OC43)	OC43	$1.1x10^5$	-	-	-
Human coronavirus (229E)	229E	$1.1x10^5$	-	-	-
Cytomegalovirus	68-1	$1x10^5$	-	-	-
Enterovirus, H. coxsackievirus B4	J.V.B.	$1x10^5$	-	-	-
Epstein Barr Virus	B95-8	$6.2x10^6$	-	-	-
Human Parainfluenza Type 1	C35	$6.2x10^6$	-	-	-
Human Parainfluenza Type 2	Greer	$2x10^5$	-	-	-
Human Parainfluenza Type 3	C243	$1.1x10^7$	-	-	-
Measles	Edmonston	$1.1x10^5$	-	-	-
Mumps virus	Enders	$1.1x10^6$	-	-	-
Human Parainfluenza Type 4a	M-25	$1.1x10^5$	-	-	-
Human Rhinovirus Type 1A	2060	$1.1x10^5$	-	-	-
Human Metapneumovirus	B-1	$5.6x10^4$	-	-	-
			Influenza A	**Influenza B**	**RSV**
Bacteria		**cfu/mL**			
Acinetobacter baumannii		$3x10^6$	-	-	-
Bordetella bronchiseptica		$3x10^5$	-	-	-
Chlamydia pneumoniae	CM-1	$1.6x10^6$	-	-	-
Corynebacterium pseudodiphtheriticum		$8.2x10^6$	-	-	-
Escherichia coli		$1.5x10^6$	-	-	-
Haemophilus influenzae		$1x10^7$	-	-	-
Klebsiella pneumoniae		$5x10^5$	-	-	-
Lactobacillus acidophilus		$2.1x10^7$	-	-	-
Legionella pneumophila		$2.1x10^6$	-	-	-
Listeria innocua		$1.2x10^7$	-	-	-
Moraxella catarrhalis		$1x10^8$	-	-	-
Neisseria gonorrhoeae		$5x10^6$	-	-	-
Neisseria meningitidis		$1x10^5$	-	-	-
Proteus vulgaris		$6x10^8$	-	-	-
Pseudomonas aeruginosa		$6.9x10^6$	-	-	-
Staphylococcus aureus		$6.1x10^6$	-	-	-
Staphylococcus epidermidis		$7.3x10^6$	-	-	-
Streptococcus pneumoniae		$9x10^6$	-	-	-
Streptococcus agalactiae		$2x10^6$	-	-	-
Streptococcus pyogenes		$2.5x10^6$	-	-	-
Streptococcus salivarius		$8.3x10^6$	-	-	-
Mycoplasma pneumoniae		$3x10^6$	-	-	-
*Mycobacterium tuberculosis, attenuated**		$2.6x10^6$	-	-	-

Analytical specificity studies were performed to assess potential cross-reactivity of the VRNAT with common respiratory pathogens and other microorganisms commonly present in specimens collected from the nasopharynx. A total of 38 organisms of interest were identified as respiratory pathogens with which the majority of the population may be infected. These included 15 viruses and 23 bacterial strains which were propagated and the titers were determined. To assess cross-reactivity, each organism was diluted in a sample matrix (Universal Transport Medium, Copan) to the concentration listed in the table below and taken through all the steps of the VRNAT protocol.

None of the organisms tested interfered with the internal controls. For all the organisms tested in the VRNAT for cross-reactivity 'Not Detected' calls were made by the Verigene Reader for each of the three viruses in the VRNAT, Influenza A, Influenza B, and RSV.. No cross-reactivity was observed with the common respiratory pathogens and organisms infecting the majority of the population.

G. Competitive Inhibition

Competitive inhibition in the VRNAT was assessed by using samples containing 2 viruses where one virus was ~3 logs higher than the other virus. Typically, the virus at the lower concentration was held near its limit of detection. Thus, each virus in the panel was tested against the remaining two viruses at higher concentrations and the Verigene result was used as a measure for assessing inhibition. The following strains were employed in the study: Influenza A/Wisconsin/67/05 (H3N2); Influenza B/ Florida/04/2006; RSV A Strain A2; RSV B Strain Wash/18537/62.

In the binary combinations tested in these studies there was no evidence of competitive inhibition.

Low	TCID50/mL	High	TCID50/mL	Influenza A	Influenza B	RSV
Influenza A	5	Influenza B	50000	+	+	-
Influenza A	5	RSV A	50000	+	-	+
Influenza A	5	RSV B	50000	+	-	+
Influenza B	10	Influenza A	50000	+	+	-
Influenza B	10	RSV A	50000	-	+	+
Influenza B	10	RSV B	50000	-	+	+
RSV A	25	Influenza A	50000	+	-	+
RSV A	25	Influenza B	50000	-	+	+
RSV B	10	Influenza A	50000	+	-	+
RSV B	10	Influenza B	50000	-	+	+

H. Fresh vs. Frozen

A set of 92 respiratory specimens were collected prospectively during the 2007-2008 respiratory season and tested to compare the performance of the Verigene® Respiratory Virus Nucleic Acid Test using fresh and frozen samples. An aliquot of the specimens were tested by using VRNAT while in the fresh, unfrozen state for the presence of Flu A/B and RSV. For each sample, the remaining sample volume was stored frozen at ≤ -70 °C for a minimum of four (4) months. The frozen samples were thawed and re-tested by using the VRNAT. The Verigene test results for the fresh samples and the corresponding frozen samples were compared to assess the VRNAT performance.

Of the 92 samples tested in the fresh and frozen state three (3) samples were excluded from the analysis as there was insufficient volume to perform tests for both fresh and frozen samples. A total of 89 paired samples were included in the results analyses, of which 27 paired samples gave positive 'Detected' results for one of the three

viruses detected by VRNAT. Another 59 paired fresh and frozen samples gave 'Not Detected' calls for all three viruses. In all, there were three (3) discordant results as three (3) of the frozen samples that generated RSV-positive calls gave 'Not Detected' calls for the corresponding fresh samples.

Fresh vs. Frozen Comparison		FRESH					
		Influenza A		Influenza B		RSV	
		Detected	Not Detected	Detected	Not Detected	Detected	Not Detected
FROZEN	Detected	5	0	14	0	8	3
	Not Detected	0	84	0	75	0	78
	Positive % Agreement	100% (95%CI=56.6%-100%)		100% (95%CI=78.5%-100%)		100% (95%CI=67.6%-100%)	
	Negative % Agreement	100% (95%CI=95.6%-100%)		100% (95%CI=91.5%-100%)		96.30% (95%CI=89.7%-98.7%)	

The discordant results (three paired samples negative for all analytes when tested fresh but positive for RSV when tested after they were stored frozen) were further analyzed. The RNA isolated from the fresh and frozen samples was subjected to bi-directional sequencing. Sequencing results confirmed that two (2) of the three tested sample pairs were RSV-positive in fresh and frozen states; repeated attempts to amplify the RNA from the fresh and frozen aliquots of the third sample failed to yield an amplicon needed for sequencing.

I. Carry-Over / Cross-Contamination Study

Based on the collective data, there was no evidence of cross-contamination from any of the test steps including sample preparation on the NucliSens EasyMAG (bioMérieux), RT-PCR step, and the Verigene Test. Moreover, there was no evidence of any cross over within the Verigene Processor modules when high titer samples were alternated with low titer samples.

J. Interferences

The potential inhibitory effect of interfering substances or interferents that may be encountered in nasopharyngeal specimens on the Verigene Respiratory Nucleic Acid Test (VRNAT) was assessed. The viral strains and the titers used in the studies are listed in Table 1. The interferents and the amount employed in the tests are described in Table 2.

Table 1

Viral Strain	Concentration (TCID50/mL)
Influenza A/Wisconsin/67/05 (H3N2)	10
Influenza B/Maryland/1/59	10
RSV A Strain Long	50
RSV B Strain B-1 Wild Type (B WV/14617/85)	10

None of the potential interferents, except the Flu vaccine, affected the VRNAT performance making the correct 'Detected' calls for the panel viruses present in samples containing the interferents. Serial dilutions of the injectable Flu vaccine, comprising inactivated influenza virus vaccine strains of Influenza A (Flu A/Solomon Islands/3/2006; Flu A/Wisconsin/67/2005) and Influenza B (Flu B/Malaysia/2506/2004) was tested in the VRNAT in the absence of Influenza A/B and RSV A/B strains. The amount of virus in the original vaccine suspension,

distributed equally among the three strains, was $9.0x10^{-5}$ g/mL. Based on this value, it was determined that Influenza A was not detectable below $4.5x10^{-11}$ g/mL and Influenza B was not detectable below $4.5x10^{-13}$ g/mL.

Table 2

Active Interferent	Source	Amount
No Interferent	Universal Transport Media	Not Applicable
Human Blood	Human Blood	5% v/v
Zanamivir	Relenza	3 mg/mL
Oseltamivir	Tamiflu	15 mg/mL
NaCl	Saline Nasal Spray	10% v/v
Mucin	MucinexD	4 mg/mL
Phenylephrine	Neo-Synephrine	10% v/v of sample
Oxymetazoline	Sinex	10% v/v of sample
Budesonide	Pulmicort	40 µg/mL
Fluticasone propionate	Flonase	2.5% v/v
Homeopathic Remedies		
Luffa opperculata	Similason Sinus Relief	1% v/v of sample
Sulfur	Boiron	4.5 mg/mL
Galphimia Glauca	Boiron	4.5 mg/mL
Histaminum Hydrochloricum	Boiron	4.5 mg/mL
Beclomethasone dipropionate	Beclomethasone dipropionate	210 µg/mL
Flunisolide	Flunisolide	125 µg/mL
Triamcinolone acetonide	Triamcinolone acetonide	27.5 µg/mL
Guaifenesin	Robitussin	2 mg/mL
Diphenhydramine hydrochlorine	Benadryl	0.5 mg/mL
Dextromethorphan hydrobromide	Delsym	1 mg/mL
Pseudoephedrine hydrochloride	Sudafed	20 µg/mL
Benzocoaine	Cepacol	5 mg/mL
Menthol	Halls	5 mg/mL
Tobramycin	Tobramycin	1.5 mg/mL
Mupirocin	Mupirocin	10 mg/mL
Amoxicillin	Amoxicillin	1 mg/mL
Flu Vaccine*	Afluria	Dilutions: $4.5x10^{-9}$ g/mL – $4.5x10^{-15}$ g/mL

* Flu Vaccine was tested in the absence of the Influenza A/B and RSV A/B strains.

Beclomethasone dipropionate | 210 µg/mL

[i] Whiley D.M., *et al.* Nucleic acid amplification testing for *Neisseria gonorrhoeae* an ongoing challenge. *J Mol. Diag*, 2006; 8: 3-15.

[ii] Hamilton, M.S., *et al.* High frequency of competitive inhibition in the Roche Cobas AMPLICOR multiplex PCR for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. *J Clin. Microbiol.* 2002; 40: 4394.

[iii] Bialasiewicz S., *et al.* Impact of competitive inhibition and sequence variation upon the sensitivity of Malaria PCR. *J. Clin. Microbiol.* 2007; 45: 1621-1623.

[iv] Whiley D.M., *et al.* Comparison of three in-house multiplex PCR assays for the detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* using real-time and conventional detection methodologies. *Pathology* 2005; 37: 364-370.

[v] Taton,T.A., Mirkin,C.A. and Letsinger,R.L. Scanometric DNA array detection with nanoparticle probes. *Science* 2000, 289, 1757–1760.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

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MAY - 1 2009

Gregory W. Shipp, MD
Chief Medical Officer
Vice President, Medical and Regulatory Affairs
and Quality Assurance
Nanosphere, Inc
4088 Commercial Avenue
Northbrook, IL 60062

Re: K083088
Trade/Device Name: Verigene® Respiratory Virus Nucleic Acid Test
Regulation Number: 21 CFR 866.3980
Regulation Name: Respiratory viral panel multiplex nucleic acid assay
Regulatory Class: Class II
Product Code: OCC, NSU
Dated: December 26, 2008
Received: April 7, 2009

Dear Dr. Shipp:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820).

This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801), please contact the Office of In Vitro Diagnostic Device Evaluation and Safety at 240-276-0450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). For questions regarding postmarket surveillance, please contact CDRH's Office of Surveillance and Biometric's (OSB's) Division of Postmarket Surveillance at 240-276-3474. For questions regarding the reporting of device adverse events (Medical Device Reporting (MDR)), please contact the Division of Surveillance Systems at 240-276-3464. You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (240) 276-3150 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Sally A. Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and
Radiological Health

Enclosure

Indication for Use

510(k) Number (if known): K083088

Device Name: Verigene® Respiratory Virus Nucleic Acid Test

Indication For Use:

The Verigene® Respiratory Virus Nucleic Acid Test is a qualitative multiplex *in vitro* diagnostic test for the detection and identification of Influenza A Virus, Influenza B Virus, and Respiratory Syncytial Virus (RSV) nucleic acids purified from nasopharyngeal swab specimens obtained from patients symptomatic for viral upper respiratory infection. The test is intended to be used on the Verigene® System as an aid in the differential diagnosis of Influenza A, Influenza B, and RSV infections. The test is not intended to detect Influenza C virus.

Negative results do not preclude influenza virus or RSV infection and should not be used as the sole basis for treatment or other management decisions. It is recommended that negative test results be confirmed by culture.

Performance characteristics for Influenza A Virus were established when Influenza A/H3 and A/H1 were the predominant Influenza A viruses in circulation. When other Influenza A viruses are emerging, performance characteristics may vary.

If infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent Influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

Prescription Use __X__ (21 CFR Part 801 Subpart D) And/Or Over the Counter Use ____. (21 CFR Part 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE; CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Device Evaluation and Safety (OIVD)

Division Sign-Off
Office of In Vitro Diagnostic Device
Evaluation and Safety